REMARKS

Following entry of this amendment, claims 16-20, 24-30, 33-42, and 44-55 will be pending in this application. Claim 43 is canceled without prejudice; claims 18, 20, 33, 34, and 37-42 are currently amended to add recitations of "whole" organ throughout and recitations of specific organs to be transplanted; and new claims 46-55 are added. Support for the amendments and new claims can be found throughout the specification and claims as originally filed, e.g., at paragraph [0022] (restenosis) and [0028] (hepatitis). No new matter has been added.

New claims 46-53 are properly within the elected group and species of transplanting an organ into a recipient and administering to the recipient a pharmaceutical composition comprising nitric oxide and a pharmaceutical composition comprising carbon monoxide. Skin is commonly understood to be an organ. See, e.g., Alberts et al., Molecular Biology of the Cell, 4th Ed., pp. 1259-60 and Figure 22-1, submitted herewith as Exhibit A ("The skin can be viewed as a large organ composed of two main tissues: the epidermis and the underlying connective tissue, which consists of the dermis and the hypodermis."). Additionally, claims directed to transplantation of a skin organ have already been examined. See canceled claim 43, which recited that the organ being transplanted is skin.

35 U.S.C. § 112, second paragraph

Claims 18-20, 24-30, and 33-45 were rejected as allegedly indefinite for later uses of the term "organ" following the phrase "whole organ." Applicants maintain that the use of the term "organ" is clear from the specification.

> Consistent with the well-established axiom in patent law that a patentee or applicant is free to be his or her own lexicographer, a patentee or applicant may use terms in a manner contrary to or inconsistent with one or more of their ordinary meanings if the written description clearly redefines the terms.

MPEP 2173.05(a) II. Applicants further submit that the term "organ" is clear in subsequent uses from antecedent basis as referring to the "whole organ." However, solely to further prosecution, applicants have amended claims 18, 20, 33, 34, and 37-41 to recite a "whole" organ at every instance as suggested by the Office. Applicants respectfully request withdrawal of the rejection.

35 U.S.C. 112, first paragraph

Claims 18-20, 24-30, and 33-45 were rejected as allegedly not enabled. Applicants respectfully traverse on the grounds that, in view of the well-developed state of the prior art regarding organ transplantation, the guidance in the specification regarding administration of nitric oxide (NO) and carbon monoxide (CO), the level of skill in the transplant field, and what was known in the art regarding NO and CO, a person of ordinary skill in the art would be able to carry out the claimed methods without undue experimentation.

In the interest of advancing prosecution of this application, applicants have amended claim 16 to recite that the organ is a liver, kidney, heart, pancreas, lung, or small intestine. Additionally, applicants have separated claims relating to skin transplantation to new claim 46 and its dependent claims. As demonstrated in the Reply submitted April 21, 2008, the state of the art with regard to transplantation of these organs was developed and robust at the time of filing.

The Office action states at pages 3-4 that:

> Pharmaceutical therapies are unpredictable for the following reasons: (1) therapeutic compositions may be inactivated before producing an effect; (2) the therapeutic composition may not reach the target area; (3) other functional properties, known or unknown, may make the therapeutic composition unsuitable for *in vivo* therapeutic use. See page 1338, footnote 7 of *Ex parte Aggarwal*, 23 USPQ2d 1334 (PTO Bd. App. & Inter. 1992).

This footnote of *Ex parte Aggarwal* cited by the Office in support of the present rejection relates to the examiner's reasoning to reject the claims in that case. Further, the examiner's reasoning for rejecting the claims in *Ex parte Aggarwal* was specific to the therapeutic protein at issue in that case. The present Office Action has not explained why those same reasons apply to the particular therapeutic compositions used in the presently claimed methods. Applicants submit that they do not. Both NO and CO have been administered clinically to humans. For example, NO (INOMax®) has been approved for use in treating pulmonary hypertension in newborns (see Exhibit B), and CO is currently in Phase II clinical trials for use in kidney transplantation (see Exhibit C). Clearly, the art recognizes that both CO and NO are suitable for *in vivo* therapeutic use. See also US 7,238,469 (administration of CO to transplant donor; already of record);

US 6,391,895 (administration of NO-releasing compounds for transplantation surgery); US 6,656,452 (administration of NO for transplantation surgery; already of record); and US 6,811,768 (administration of NO for non-pulmonary inflammation, including transplant rejection; already of record). Applicants respectfully submit that the examiner's rationale in *Ex parte Aggarwal* is not relevant to the NO and CO (or any other composition recited in the claims).

The Office continues to point to Calabrese et al. (Xenotransplantation 10:488, Abstract, 2003) and Cozzi et al. (Xenotransplantation 10:528, Abstract, 2003) to support its proposition that CO treatment is unpredictable. These describe pig-to-primate kidney transplantations where CO was administered only to the donor prior to transplantation. CO did not significantly extend graft survival (see Cozzi et al.), although the treatment of the donor did have a positive effect to reduce apoptosis in this extremely stringent xenotransplantation model (see Calabrese et al.). Regardless, these abstracts describe CO treatment of the graft donor, not the recipient as recited in the claimed methods currently under examination. The Office contends that these abstracts "appear to be the closest animal models of the transplantation method claimed found in the literature." Applicants respectfully disagree. In fact, the literature provides many examples where CO was administered to transplant recipients. For example, as described in the previous reply, U.S. Pat. No. 7,238,469 discloses that administration of CO gas to a transplant recipient enhances survival of a transplanted organ (heart) or cells (islet cells) in the recipient (see col. 24-42). Song et al. (Am. J. Pathol., 163:231-242, 2003; already of record) demonstrated that CO administration to rat lung transplant recipients reduced apoptosis and inflammation characteristic of acute rejection. Nakao et al. (Surgery 134:285-292, 2003; already of record) discloses that administration of CO to rat intestine transplant recipients increased survival to 100% from 58% for air-treated controls. Otterbein et al. (Nature Medicine 9:183-90, 2003; already of record) shows that CO administration to rat recipients of aorta grafts inhibited intimal hyperplasia characteristic of graft rejection. Similarly, Ramlawi et al. (J. Surg. Res. 138:121-127, 2007; already of record) demonstrates that CO administration prevents intimal hyperplasia in a pig model. See also Nakao et al., "Protective effect of carbon monoxide in transplantation," J. Cell. Mol. Med. 10:650-671 (2006), which reviews the results of several publications describing the protective effect of CO in transplantation. Also, as mentioned above, CO is currently in Phase II

clinical trials for administration to kidney transplantation recipients (see Exhibit C). In view of the wealth of information in the literature describing successful treatment by administration of CO to transplant recipients, Calabrese et al. and Cozzi et al. do not represent the closest animal models in the literature to the methods currently under examination. Rather, the bulk of the art acknowledges the usefulness of CO in transplantation methods.

NO has also been recognized as useful in transplantation methods. U.S. Pat. No. 6,391,895; 6,656,452 (already of record); and 6,811,768 (already of record) all describe and claim the use of NO or NO-releasing agents in transplantation methods. Haraldsson et al. (Chest 114:780-786, 1998; already of record), Kanno et al. (Circulation 101:2742-48, 2000; already of record), Rajek et al. (Anesth. Analg. 90:523-530, 2000; already of record), and the post-filing date publication Dietl et al. (Pharmacol. Rep. 58 Suppl:145-152, 2006) demonstrate that NO is useful in heart transplant methods or to protect against ischemia/reperfusion injury in the heart. Meyer et al. (Chest 113:1360-1371, 1998; already of record) touts the potential of NO in lung transplantation. The post-filing date publication Lang et al. (J. Clin. Invest. 117:2583-91, 2007) demonstrates that administration of inhaled NO to liver transplant recipients improved liver function and significantly reduced the time of hospitalization. The Meade et al. reference (Am. J. Respir. Crit. Care Med. 167:1483-89, 2003) cited by the Office may have had several experimental problems, as admitted by its authors. The authors state that any of improper timing of NO administration, improper NO dosage, blinding of the experiment, or a small sample size may have led to the absence of effect seen in their experiment (p. 1488). The majority of the art appears to acknowledge the usefulness of NO in transplantation methods.

While the examples section of the present specification does not provide data wherein transplant recipients were dosed with NO and CO, as the office seems to require at this time (and which applicants submit is not required for patentability of the claimed methods), it does provide data that demonstrate the interrelationship between CO/HO-1 and NO/iNOS. Using *in vitro* and *in vivo* models of inflammation in mice, applicants determined that increased expression of iNOS is involved in providing the protective effects of CO, whereas the protective effects of NO similarly involve up-regulation of HO-1 expression. When protection from cell death is initiated by CO, NO production and HO-1 activity are each important for the protective effect. These

results showed for the first time an essential synergy between CO and NO in providing cytoprotection.

Further, a beneficial effect of applicants' methods of co-administration of NO and CO was confirmed in Raman et al., 2006, "Inhaled carbon monoxide inhibits intimal hyperplasia and provides added benefit with nitric oxide," J. Vasc. Surg. 44:151-158 (already of record). In a porcine model of intimal hyperplasia, the combined administration of CO (administered as an inhaled gas) and NO (via expression of a vector encoding inducible nitric oxide synthase (iNOS)) provided additional protection as compared to either CO or NO administered singly. Because intimal hyperplasia is a hallmark of chronic transplant rejection,[1] these results are clearly relevant to the claimed transplantation methods.

In view of the well-developed state of the prior art regarding organ transplantation, the guidance in the specification regarding administration of nitric oxide (NO) and carbon monoxide (CO), the level of skill in the transplant field, and what was known in the art regarding NO and CO , a person of ordinary skill in the art would be able to carry out the methods recited in the claims without undue experimentation. Applicants therefore request reconsideration and withdrawal of the rejection for alleged lack of enablement.

[1] See, e.g., Nieuwenhuis et al., "Chronic allograft rejection associated vasculopathy and synthetic biodegradable vascular grafts: a lesson to learn?" Crit. Rev. Immunol., 20:85-88, 2000; Kouwenhoven et al., "Etiology and pathophysiology of chronic transplant dysfunction," Transpl. Int. 13:385-401, 2000; and U.S. Pat. No. 7,238,429, col. 42, ll. 20-52.

CONCLUSION

Applicants submit that all pending claims are in condition for allowance, which action is requested. Enclosed is a Petition for Extension of Time along with the required fee. Please apply any other required charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 13681-0012001.

Respectfully submitted,

Date: February 11, 2009

/RSMcQuade/
Ryan S. McQuade, Ph.D.
Reg. No. 61,358

Fish & Richardson P.C.
Customer No. 26161
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

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Exhibit A

MOLECULAR BIOLOGY OF

THE CELL

fourth edition

Bruce Alberts

Alexander Johnson

Julian Lewis

Martin Raff

Keith Roberts

Peter Walter

GS Garland Science
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Artistic and Scientific Direction: Peter Walter
Narrated by: Julie Theriot
Production, Design, and Development: Mike Morales

Bruce Alberts received his Ph.D. from Harvard University and is President of the National Academy of Sciences and Professor of Biochemistry and Biophysics at the University of California, San Francisco. **Alexander Johnson** received his Ph.D. from Harvard University and is a Professor of Microbiology and Immunology at the University of California, San Francisco. **Julian Lewis** received his D.Phil. from the University of Oxford and is a Principal Scientist at the Imperial Cancer Research Fund, London. **Martin Raff** received his M.D. from McGill University and is at the Medical Research Council Laboratory for Molecular Cell Biology and Cell Biology Unit and in the Biology Department at University College London. **Keith Roberts** received his Ph.D. from the University of Cambridge and is Associate Research Director at the John Innes Centre, Norwich. **Peter Walter** received his Ph.D. from The Rockefeller University in New York and is Professor and Chairman of the Department of Biochemistry and Biophysics at the University of California, San Francisco, and an Investigator of the Howard Hughes Medical Institute.

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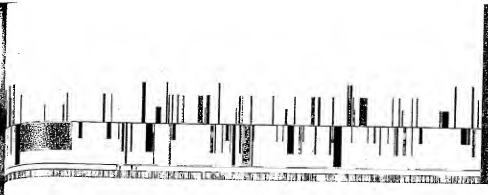
Front cover Human Genome: Reprinted by permission from *Nature*, International Human Genome Sequencing Consortium, 409:860–921, 2001 © Macmillan Magazines Ltd. Adapted from an image by Francis Collins, NHGRI; Jim Kent, UCSC; Ewan Birney, EBI; and Darryl Leja, NHGRI; showing a portion of Chromosome 1 from the initial sequencing of the human genome.

Back cover In 1967, the British artist Peter Blake created a design classic. Nearly 35 years later Nigel Orme (illustrator), Richard Denyer (photographer), and the authors have together produced an affectionate tribute to Mr Blake's image. With its gallery of icons and influences, its assembly created almost as much complexity, intrigue and mystery as the original. *Drosophila*, *Arabidopsis*, Dolly and the assembled company tempt you to dip inside where, as in the original, "a splendid time is guaranteed for all." (Gunter Blobel, courtesy of The Rockefeller University; Marie Curie, Keystone Press Agency Inc; Darwin bust, by permission of the President and Council of the Royal Society; Rosalind Franklin, courtesy of Cold Spring Harbor Laboratory Archives; Dorothy Hodgkin, © The Nobel Foundation, 1964; James Joyce, etching by Peter Blake; Robert Johnson, photo booth self-portrait early 1930s, © 1986 Delta Haze Corporation all rights reserved, used by permission; Albert L. Lehninger, (unidentified photographer) courtesy of The Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions; Linus Pauling, from Ava Helen and Linus Pauling Papers, Special Collections, Oregon State University; Nicholas Poussin, courtesy of ArtToday.com; Barbara McClintock, © David Micklos, 1983; Andrei Sakharov, courtesy of Elena Bonner; Frederick Sanger, © The Nobel Foundation, 1958.)



22

HISTOLOGY: THE LIVES AND DEATHS OF CELLS IN TISSUES

Cells evolved originally as free-living individuals, but the cells that matter most to us, as human beings, are specialized members of a multicellular community. They have lost features needed for independent survival and acquired peculiarities that serve the needs of the body as a whole. Although they share the same genome, they are spectacularly diverse: more than 200 different cell types are traditionally recognized in the human body (see our web site for a list). These collaborate with one another to form a multitude of different tissues, arranged into organs performing widely varied functions. To understand them, it is not enough to analyze them in a culture dish: we need also to know how they live, work, and die in their natural habitat.

In Chapters 7 and 21, we saw how the various cell types become different in the embryo and how cell memory and signals from their neighbors enable them to remain different thereafter. In Chapter 19, we discussed the building technology of multicellular tissues—the devices that bind cells together and the extracellular materials that give them support. In this chapter, we consider the functions and lifestyles of the specialized cells in the adult body of a vertebrate. We describe how cells work together to perform their tasks, how new specialized cells are born, how they live and die, and how the architecture of tissues is preserved despite the constant replacement of old cells by new.

We examine these topics through a series of examples—some chosen because they illustrate important general principles, others because they highlight favorite objects of study, still others because they pose intriguing problems that cell biology has yet to solve.

EPIDERMIS AND ITS RENEWAL BY STEM CELLS

To support its specialized functions, the skin has basic requirements that must be satisfied for almost every tissue. It needs mechanical strength, largely provided by a supporting framework of extracellular matrix, mainly secreted by *fibroblasts*. It needs a blood supply to bring nutrients and oxygen and remove waste products and carbon dioxide, and this requires a network of blood vessels, lined with

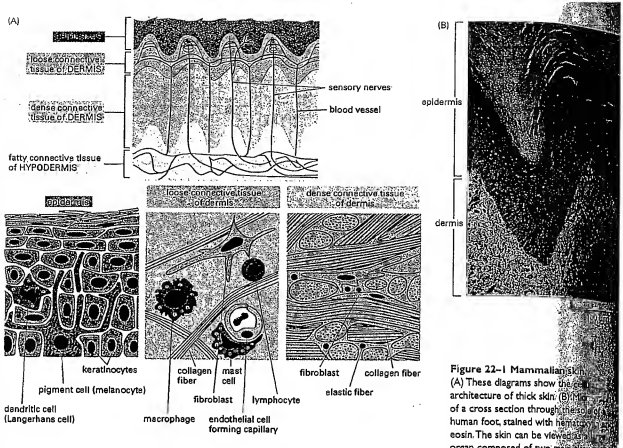


Figure 22–1 Mammalian skin. (A) These diagrams show the cellular architecture of thick skin. (B) Micrograph of a cross section through the skin of a human foot, stained with hematoxylin and eosin. The skin can be viewed as an organ composed of two main tissues: the epidermis and the underlying connective tissue, which consists of the dermis and the hypodermis. Each tissue is composed of a variety of cell types. The dermis and hypodermis are richly supplied with blood vessels and nerves. Some nerves also extend into the epidermis.

endothelial cells. These vessels also provide access routes for cells of the immune system to provide defenses against infection: *macrophages* and *dendritic* cells phagocytose invading pathogens and help activate lymphocytes, which mediate more sophisticated adaptive immune system responses (discussed in Chapter 24). *Nerve fibers* are needed too, to convey sensory information from the tissue to the central nervous system, and to deliver signals in the opposite direction for glandular secretion and smooth muscle contraction.

Figure 22–1 illustrates the architecture of the tissue and shows how it makes provision for all these support services. Skin consists of two main parts: an epithelium, the *epidermis*, lying outermost, and beneath this a layer of connective tissue, which includes the tough collagen-rich *dermis* (from which leather is made) and the underlying fatty *subcutaneous layer* or *hypodermis*. In the skin, as elsewhere, the connective tissue, with vessels and nerves running through it, is responsible for most of the general supportive functions listed above.

The defining component of the skin—the specialized tissue that is peculiar to this organ, even though not the major part of its bulk—is the epidermis. This has a simple organization, and it provides a beautiful introduction to the way in which tissues of the adult body are continually renewed, through processes similar to those that operate in the embryo. We return to connective tissues later.

Epidermal Cells Form a Multilayered Waterproof Barrier

The **epidermis** suffers more direct, frequent, and damaging encounters with the external world than any other tissue in the body. Its need for repair and renewal is central to its organization.

The epidermis is a multilayered *(stratified)* epithelium composed largely of *keratinocytes* (so named because their characteristic differentiated activity is the synthesis of intermediate filament proteins called keratins, which give the epidermis its toughness) (Figure 22–2). These cells change their appearance from one layer to the next. Those in the innermost layer, attached to an underlying

INOmax® (nitric oxide) for inhalation
100 and 800 ppm (parts per million)

DESCRIPTION

INOmax (nitric oxide gas) is a drug administered by inhalation. Nitric oxide, the active substance in INOmax, is a pulmonary vasodilator. INOmax is a gaseous blend of nitric oxide and nitrogen (0.08% and 99.92%, respectively for 800 ppm; 0.01% and 99.99%, respectively for 100 ppm). INOmax is supplied in aluminum cylinders as a compressed gas under high pressure (2000 pounds per square inch gauge [psig]).

The structural formula of nitric oxide (NO) is shown below:

CLINICAL PHARMACOLOGY

Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3',5'-monophosphate, which then leads to vasodilation. When inhaled, nitric oxide produces pulmonary vasodilation.

INOmax appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better ventilated areas of the lung, redistributing pulmonary blood flow away from lung regions with low ventilation/perfusion (V/Q) ratios toward regions with normal ratios.

Effects on Pulmonary Vascular Tone in PPHN

Persistent pulmonary hypertension of the newborn (PPHN) occurs as a primary developmental defect or as a condition secondary to other diseases such as meconium aspiration syndrome (MAS), pneumonia, sepsis, hyaline membrane disease, congenital diaphragmatic hernia (CDH), and pulmonary hypoplasia. In these states, pulmonary vascular resistance (PVR) is high, which results in hypoxemia secondary to right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale. In neonates with PPHN, INOmax improves oxygenation (as indicated by significant increases in PaO_2).

PHARMACOKINETICS

The pharmacokinetics of nitric oxide has been studied in adults.

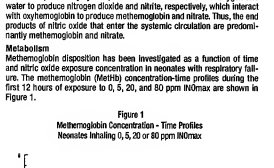
Uptake and Distribution

Nitric oxide is absorbed systemically after inhalation. Most of it traverses the pulmonary capillary bed where it combines with hemoglobin that is 60% to 100% oxygen-saturated. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. At low oxygen saturation, nitric oxide can combine with deoxyhemoglobin to transiently form nitrosylhemoglobin, which is converted to nitrogen oxides and methemoglobin upon exposure to oxygen. Within the pulmonary system, nitric oxide can combine with oxygen and water to produce nitrogen dioxide and nitrite, respectively, which interact with oxyhemoglobin to produce methemoglobin and nitrate. Thus, the end products of nitric oxide that enter the systemic circulation are predominantly methemoglobin and nitrate.

Metabolism

Methemoglobin disposition has been investigated as a function of time and nitric oxide exposure concentration in neonates with respiratory failure. The methemoglobin (MetHb) concentration-time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm INOmax are shown in Figure 1.

Figure 1
Methemoglobin Concentration - Time Profiles
Neonates Inhaling 0, 5, 20 or 80 ppm INOmax



Methemoglobin concentrations increased during the first 8 hours of nitric oxide exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5 ppm and 20 ppm INOmax groups, but reached approximately 5% in the 80 ppm INOmax group. Methemoglobin levels >7% were attained only in patients receiving 80 ppm, where they comprised 25% of the group. The average time to reach peak methemoglobin was 10 ± 9 (SD) hours (median, 8 hours) in these 13 patients; but one patient did not exceed 7% until 40 hours.

Elimination

Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.

CLINICAL TRIALS

The efficacy of INOmax has been investigated in term and near-term newborns with hypoxic respiratory failure resulting from a variety of etiologies. Inhalation of INOmax reduces the oxygenation index (OI)—mean airway pressure in cm H_2O x fraction of inspired oxygen concentration (FiO_2) x 100 divided by systemic arterial concentration in mm Hg (PaO_2)) and increases PaO_2 (See CLINICAL PHARMACOLOGY).

EXHIBIT B

NINOS study

The Neonatal Inhaled Nitric Oxide Study (NINOS) group conducted a double-blind, randomized, placebo-controlled, multicenter trial in 235 neonates with hypoxic respiratory failure. The objective of the study was to determine whether inhaled nitric oxide would reduce the occurrence of death and/or initiation of extracorporeal membrane oxygenation (ECMO) in a prospectively defined cohort of term or near-term neonates with hypoxic respiratory failure unresponsive to conventional therapy. Hypoxic respiratory failure was caused by meconium aspiration syndrome (MAS; 49%), pneumonia/sepsis (21%), idiopathic primary pulmonary hypertension of the newborn (PPHN; 17%), or respiratory distress syndrome (ARDS; 11%). Infants ≤14 days of age (mean, 1.7 days) with a mean PaO_2 of 46 mm Hg and a mean oxygenation index (OI) of 43 cm H_2O / mm Hg were initially randomized to receive 100% O_2 with (n=114) or without (n=121) 20 ppm nitric oxide for up to 14 days. Response to study drug was defined as a change from baseline in PaO_2 30 minutes after starting treatment (full response = >20 mm Hg, partial = 10–20 mm Hg, no response = <10 mm Hg). Neonates with a less than full response were evaluated for a response to 80 ppm nitric oxide or control gas. The primary results from the NINOS study are presented in Table 1.

Table 1
Summary of Clinical Results from NINOS Study

	Control (n=121)	NO (n=114)	P value
Death or ECMO*,†	77 (64%)	52 (46%)	0.006
Death	20 (17%)	16 (14%)	0.60
ECMO	66 (55%)	44 (39%)	0.014

* Extracorporeal membrane oxygenation
† Death or need for ECMO was the study's primary end point

Although the incidence of death by 120 days of age was similar in both groups (NO, 14%; control, 17%), significantly fewer infants in the nitric oxide group required ECMO compared with controls (39% vs. 55%, p = 0.014). The combined incidence of death and/or initiation of ECMO showed a significant advantage for the nitric oxide treated group (46% vs. 64%, p = 0.006). The nitric oxide group also had significantly greater increases in PaO_2 and greater decreases in the OI and the alveolar-arterial oxygen gradient than the control group (p<0.001 for all parameters). Significantly more patients had at least a partial response to the initial administration of study drug in the nitric oxide group (66%) than the control group (26%, p<0.001). Of the 125 infants who did not respond to 20 ppm nitric oxide or control, similar percentages of NO-treated (18%) and control (20%) patients had at least a partial response to 80 ppm nitric oxide for inhalation or control drug, suggesting a lack of additional benefit for the higher dose of nitric oxide. No infant had study drug discontinued for toxicity. Inhaled nitric oxide had no detectable effect on mortality. The adverse events collected in the NINOS trial occurred at similar incidence rates in both treatment groups (See ADVERSE REACTIONS). Follow-up exams were performed at 18–24 months for the infants enrolled in this trial. In the infants with available follow-up, the two treatment groups were similar with respect to their mental, motor, audiologic, or neurologic evaluations.

CINRGI study

This study was a double-blind, randomized, placebo-controlled, multicenter trial of 186 term and near-term neonates with pulmonary hypertension and hypoxic respiratory failure. The primary objective of the study was to determine whether INOmax would reduce the receipt of ECMO in these patients. Hypoxic respiratory failure was caused by MAS (35%), idiopathic PPHN (30%), pneumonia/sepsis (24%), or RDS (8%). Patients with a mean PaO_2 of 54 mm Hg and a mean OI of 44 cm H_2O / mm Hg were randomly assigned to receive either 20 ppm INOmax (n=97) or nitrogen gas (placebo; n=89) in addition to their ventilatory support. Patients who exhibited a PaO_2 >60 mm Hg and a pH < 7.55 were weaned to 5 ppm INOmax or placebo. The primary results from the CINRGI study are presented in Table 2.

Table 2
Summary of Clinical Results from CINRGI Study

	Placebo	INOmax	P value
ECMO *,†	51/89 (57%)	30/97 (31%)	<0.001
Death	5/89 (6%)	3/97 (3%)	0.48

* Extracorporeal membrane oxygenation
† ECMO was the primary end point of this study

Significantly fewer neonates in the INOmax group required ECMO compared to the control group (31% vs. 57%, p<0.001). While the number of deaths were similar in both groups (INOmax, 3%; placebo, 6%), the combined incidence of death and/or receipt of ECMO was decreased in the INOmax group (33% vs. 56%, p<0.001).

In addition, the INOmax group had significantly improved oxygenation as measured by PaO_2, OI, and alveolar-arterial gradient (p<0.001 for all parameters). Of the 97 patients treated with INOmax, 2 (2%) were withdrawn from study drug due to methemoglobin levels >4%. The frequency and number of adverse events reported were similar in the two study groups (See ADVERSE REACTIONS).

ARDS study

In a randomized, double-blind, parallel, multicenter study, 385 patients with adult respiratory distress syndrome (ARDS) associated with pneumonia (46%), surgery (33%), multiple trauma (26%), aspiration (23%), pulmonary contusion (18%), and other causes, with PaO_2/FiO_2 <250 mm Hg despite optimal oxygenation and ventilation, received placebo (n=193) or INOmax (n=192), 5 ppm, for 4 hours to 28 days or until weaned because of improvements in oxygenation. Despite acute improvements in oxygenation, there was no effect of INOmax on the primary endpoint of days alive and off ventilator support. These results were consistent with outcome data from a smaller dose ranging study of nitric oxide (1.25 to 80 ppm). INOmax is not indicated for use in ARDS.

INDICATIONS

INOmax, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

CONTRAINDICATIONS

INOmax should not be used in the treatment of neonates known to be dependent on right-to-left shunting of blood.

PRECAUTIONS

Rebound

Abrupt discontinuation of INOmax may lead to worsening oxygenation and increasing pulmonary artery pressure.

Methemoglobinemia

Methemoglobinemia increases with the dose of nitric oxide. In the clinical trials, maximum methemoglobin levels usually were reached approximately 8 hours after initiation of inhalation, although methemoglobin levels have peaked as late as 40 hours following initiation of INOmax therapy. In one study, 13 of 37 (35%) of neonates treated with INOmax 80 ppm had methemoglobin levels exceeding 7%. Following discontinuation or reduction of nitric oxide the methemoglobin levels returned to baseline over a period of hours.

Elevated NO_2 Levels

In one study, NO_2 levels were <0.5 ppm when neonates were treated with placebo, 5 ppm, and 20 ppm nitric oxide over the first 48 hours. The 80 ppm group had a mean peak NO_2 level of 2.6 ppm.

Drug Interactions

No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOmax has been administered with tolazoline, dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOmax on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of a carcinogenic effect was apparent, at inhalation exposures up to the recommended dose (20 ppm), in rats for 20 hr/day for up to two years. Higher exposures have not been investigated.

Nitric oxide has demonstrated genotoxicity in Salmonella (Ames Test), human lymphocytes, and after *in vivo* exposure in rats. There are no animal or human studies to evaluate nitric oxide for effects on fertility.

Pregnancy: Category C

Animal reproduction studies have not been conducted with INOmax. It is not known if INOmax can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. INOmax is not intended for adults.

Nursing Mothers

Nitric oxide is not indicated for use in the adult population, including nursing mothers. It is not known whether nitric oxide is excreted in human milk.

Pediatric Use

Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

ADVERSE REACTIONS

Controlled studies have included 325 patients on INOmax doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOmax, a result adequate to exclude INOmax mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOmax and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOmax and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

The table below shows adverse events with an incidence of at least 5% on INOmax in the CINRGI study, and that were more common on INOmax than on placebo.

ADVERSE EVENTS IN THE CINRGI TRIAL

Adverse Event	Placebo (n=89)	Inhaled NO (n=97)
Hypotension	9 *(10%)*	13 *(13%)*
Withdrawal	9 *(10%)*	12 *(12%)*
Atelectasis	8 *(9%)*	9 *(9%)*
Hematuria	5 *(6%)*	8 *(8%)*
Hyperglycemia	6 *(7%)*	8 *(8%)*
Sepsis	2 *(2%)*	7 *(7%)*
Infection	3 *(3%)*	6 *(6%)*
Stridor	3 *(3%)*	5 *(5%)*
Cellulitis	0 *(0%)*	5 *(5%)*

OVERDOSAGE

Overdosage with INOmax will be manifest by elevations in methemoglobin and NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobinemia reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOmax.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

POST-MARKETING EXPERIENCE

The following adverse events have been reported as part of the post-marketing surveillance. These events have not been reported above. Given the nature of spontaneously reported post-marketing surveillance data, it is impossible to determine the actual incidence of the events or definitively establish their causal relationship to the drug. The listing is alphabetical: dose errors associated with the delivery system; headaches associated with environmental exposure of INOmax in hospital staff; hypotension associated with acute withdrawal of the drug; hypoxemia associated with acute withdrawal of the drug, pulmonary edema in patients with CREST syndrome.

DOSAGE AND ADMINISTRATION

Dosage

The recommended dose of INOmax is 20 ppm. Treatment should be maintained up to 14 days or until the underlying oxygen desaturation has resolved and the neonate is ready to be weaned from INOmax therapy.

An initial dose of 20 ppm was used in the NINOS and CINRGI trials. In CINRGI, patients whose oxygenation improved with 20 ppm were dose-reduced to 5 ppm as tolerated at the end of 4 hours of treatment. In the NINOS trial, patients whose oxygenation failed to improve on 20 ppm could be increased to 80 ppm, but those patients did not then improve on the higher dose. As the risk of methemoglobinemia and elevated NO_2 levels increases significantly when INOmax is administered at doses >20 ppm, doses above this level ordinarily should not be used.

Administration

Additional therapies should be used to maximize oxygen delivery. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of inhaled nitric oxide have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies (see CLINICAL TRIALS).

INOmax should be administered with monitoring for PaO_2, methemoglobin, and NO_2.

The nitric oxide delivery systems used in the clinical trials provided operator-determined concentrations of nitric oxide in the breathing gas, and the concentration was constant throughout the respiratory cycle. INOmax must be delivered through a system with these characteristics and which does not cause generation of excessive inhaled nitrogen dioxide. The INOvent® system and other systems meeting these criteria were used in the clinical trials. In the ventilated neonate, precise monitoring of inspired nitric oxide and NO_2 should be instituted, using a properly calibrated analysis device with alarms. The system should be calibrated using a precisely defined calibration mixture of nitric oxide and nitrogen dioxide, such as INOcal®. Sample gas for analysis should be drawn before the Y-piece, proximal to the patient. Oxygen levels should also be measured.

In the event of a system failure or a wall-outlet power failure, a backup battery power supply and reserve nitric oxide delivery system should be available.

The INOmax dose should not be discontinued abruptly as it may result in an increase in pulmonary artery pressure (PAP) and/or worsening of blood oxygenation (PaO_2). Deterioration in oxygenation and elevation in PAP may also occur in children with no apparent response to INOmax. Discontinue/wean cautiously.

HOW SUPPLIED

INOmax (nitric oxide) is available in the following sizes:

Size D Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-002-01)

Size D Portable aluminum cylinders containing 353 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 344 liters) (NDC 64693-001-01)

Size 88 Aluminum cylinders containing 1953 liters at STP of nitric oxide gas in 800 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-002-02)

Size 88 Aluminum cylinders containing 1953 liters at STP of nitric oxide gas in 100 ppm concentration in nitrogen (delivered volume 1918 liters) (NDC 64693-001-02)

Store at 25°C (77°F) with excursions permitted between 15–30°C (59–86°F) [see USP Controlled Room Temperature].

Occupational Exposure

The exposure limit set by the Occupational Safety and Health Administration (OSHA) for nitric oxide is 25 ppm, and for NO_2 the limit is 5 ppm.

CAUTION

Federal law prohibits dispensing without a prescription.

INO Therapeutics
6 Route 173 West
Clinton, NJ 08809
USA

Exhibit C

Safety and Tolerability Study of Inhaled Carbon Monoxide in Kidney Transplant Patients

This study is currently recruiting participants.
Verified by INO Therapeutics, October 2008

Sponsored by:	**INO Therapeutics**
Information provided by:	INO Therapeutics
ClinicalTrials.gov Identifier:	NCT00531856

▶ Purpose

The purpose of this study is to evaluate the safety and tolerability of two carbon monoxide doses when administered as an inhaled gas for 1 hour in patients receiving kidney transplants.

Condition	Intervention	Phase
Kidney Transplantation	Drug: Inhaled carbon monoxide Drug: inhaled carbon monoxide	Phase II

MedlinePlus related topics: Kidney Transplantation

Drug Information available for: Carbon monoxide

U.S. FDA Resources

Study Type: Interventional
Study Design: Basic Science, Randomized, Single Blind (Subject), Placebo Control, Parallel Assignment, Safety/Efficacy Study

Official Title: A Prospective, Multicenter, Single-Blind, Placebo-Controlled, Safety and Tolerability Study of the Effects of Carbon Monoxide for Inhalation in Patients Receiving Kidney Transplants.

Further study details as provided by INO Therapeutics:

Primary Outcome Measures:

- Evaluate the safety and tolerability of three carbon monoxide dose levels when administered as an inhaled gas for 1 hour in patients receiving kidney transplants [Time Frame: 28 days] [Designated as safety issue: Yes]

Secondary Outcome Measures:

- Characterize the pharmacokinetics of the inhaled carbon monoxide; Correlate the safety parameters to inhaled carbon monoxide and COHb levels; Assess laboratory values; Assess potential markers for the incidence of delayed graft function [Time Frame: 28 days] [Designated as safety issue: No]

Estimated Enrollment: 16
Study Start Date: August 2007
Estimated Study Completion Date: March 2009
Estimated Primary Completion Date: March 2009 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
1: Experimental 5.97mg/L of carbon monoxide in 30% oxygen	Drug: Inhaled carbon monoxide 0.7 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours post transplant Drug: Inhaled carbon monoxide

	2.0 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours post transplant Drug: inhaled carbon monoxide 2.0 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours during transplant Drug: Inhaled carbon monoxide 3.0 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours during transplant
2: Placebo Comparator Oxygen 30% in Nitrogen	Drug: Inhaled carbon monoxide 0.7 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours post transplant Drug: Inhaled carbon monoxide 2.0 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours post transplant Drug: inhaled carbon monoxide 2.0 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours during transplant Drug: Inhaled carbon monoxide 3.0 mg/kg carbon monoxide/placebo over one hour administered 12-48 hours during transplant

Detailed Description:

The mechanisms by which carbon monoxide exerts its effects in preventing damage of the graft appear to vary among the models and organs with the common theme of carbon monoxide acting as a potent anti-inflammatory molecule. Carbon monoxide affects several intracellular signaling pathways. In addition, carbon monoxide generates increased levels of anti-inflammatory molecules.

Evaluate the safety and tolerability of three carbon monoxide dose levels consisting of a single 0.7 mg/kg dose and a single 2.0 mg/kg dose when administered post-operatively and a single 2.0 mg/kg dose and a single 3.0mg/kg dose when administered intra-operatively as an inhaled gas for 1 hour, by assessment of adverse events (AEs), vital signs, laboratory variables, serum carboxyhemoglobin (COHb), oxygenation, electrocardiography (ECG), and neurocognitive status in patients receiving kidney transplants.

▶ Eligibility

Ages Eligible for Study: 18 Years and older
Genders Eligible for Study: Both
Accepts Healthy Volunteers: No

Criteria

Inclusion Criteria:

- Male or female receiving a kidney transplant from any donor type
- BMI between 16 and 36 inclusive
- Spontaneously breathing (non-intubated) with supplemental oxygen standardized at 2 liters via nasal cannula
- Hemodynamically stable with a systolic arterial pressure > 90 mmHg and a heart rate < 120 beats/min
- Acceptable transplantation candidate as judged by medical history, physical exam, ECG, vital signs, clinical chemistry, hematology, and urinalysis
- Given written and verbal information and had the opportunity to ask questions about the study
- Signed informed consent to participate in the study

Exclusion Criteria:

- Exposure to any carbon monoxide source (e.g., fire, gas, or heavily polluted air) during the 48 hours prior to the study day
- Baseline blood level of COHb >2%
- Baseline hemoglobin (Hb) <10.0 g/dL
- Patients with significant underlying lung disease such as moderate or severe asthma, COPD, and interstitial lung disease
- Baseline oxygen saturation <95%
- Pregnancy or breastfeeding
- Participation in other clinical trial within 2 months prior to study drug treatment

Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT00531856

Contacts

Contact: Robert Small 908-238-6605 robert.small@ikaria.com

Locations

United States, California

Universtiy of CA, San Francisco — **Recruiting**
San Francisco, California, United States, 94143
Contact: Monica Rodriguez, RN 415-476-4022 RodriguezM@surgery.ucsf.edu
Principal Investigator: Sandy Feng, MD, PhD

United States, Illinois

Northwestern University — **Recruiting**
Chicago, Illinois, United States, 60611
Contact: Patrice Al-Saden, RN 312-503-1058 palsaden@northwestern.edu
Principal Investigator: Xunrong Luo, MD

United States, Massachusetts

Beth Israel Deaconess Medical Center — **Not yet recruiting**
Boston, Massachusetts, United States, 02215
Contact: Robyn Chudzinski, Pharm D 617-632-9841 rchudzin@caregroup.harvard.edu
Principal Investigator: Douglas W Hanto, MD

Sponsors and Collaborators

INO Therapeutics

More Information

Responsible Party:	INO Therapeutics (Robert Small, RN)
Study ID Numbers:	C201
First Received:	September 18, 2007
Last Updated:	October 21, 2008
ClinicalTrials.gov Identifier:	NCT00531856 [history]
Health Authority:	United States: Food and Drug Administration

Keywords provided by INO Therapeutics:
Kidney Transplant, Carbon Monoxide

Study placed in the following topic categories:
Carbon Monoxide

Additional relevant MeSH terms:
Antimetabolites
Molecular Mechanisms of Pharmacological Action
Pharmacologic Actions

ClinicalTrials.gov processed this record on January 09, 2009